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A STUDY OF SOME FACTORS RELATIVE TO THE PATHOGENICITY OF ACTINOMYCES SCABIES (THAXTER) GUSSOW

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
DECEMBER 1925

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[Reprinted from PHYTOPATHOLOGY, August, 1926, Vol. XVI, No. 8]

SOME FACTORS AFFECTING THE PATHOGENICITY OF ACTINOMYCES SCABIES[1]

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INTRODUCTION

Common scab of potato, *Solanum tuberosum,* caused by *Actinomyces scabies* (Thaxter) Güssow, is prevalent and destructive. All attempts effectively to control the disease have failed. The potato scab pathogene is normally a soil saprophyte, and its activities are therefore influenced by other soil microorganisms. There may be metabiotic or antibiotic relations between these organisms, and these relations, as well as the soil flora, may be altered when one or more of the physical or chemical factors of the soil are changed, but the degree of this modification and its effect on scab development is not well understood.

A review of the work done for the control of potato scab previous to 1914 has been given by Lutman and Cunningham (9). Drechsler (3) has studied the morphology of *Actinomyces,* and Waksman (17) the physiology. Others have studied various methods of reducing scab. In addition to tuber disinfection, the principal preventive measure used has been to attempt to increase the acidity of the soil. This appears to have serious economic and other limitations on many potato soils, especially those having a high initial pH value and which are highly buffered. The plowing down of green crops, particularly rye, often is recommended as a control measure for potato scab, as the decomposition of organic matter thus put into the soil is assumed to increase soil acidity.

OBJECTS OF THE INVESTIGATION

The objects of this investigation were: to study the effect of temperature, moisture, oxygen supply, soil reaction, and stimulatory substances on the germination, growth and pathogenicity of *A. scabies;* and to throw additional light on the nature of the control said to be obtained by plowing under a green crop of rye.

[1] Published with the approval of the Director as Paper No. 618 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Grateful acknowledgment is made to Professor E. C. Stakman for his assistance and helpful criticism during this study.

METHODS

A synthetic dextrose-nitrate substrate was used for all studies, except where otherwise stated, the composition being: K_2HPO_4, 1 gm; $MgSO_4$, 0.5 gm; KCl, 0.5 gm; $FeSO_4$, trace; $NaNO_3$, 2 gm; dextrose 30 gm; agar 18 gm; distilled water 1000 cc. Agar slants in loosely plugged test tubes were found preferable to petri dish cultures. It was found advisable to add the spores[3] in suspension to the surface of the solid medium. Other methods will be described when necessary. A strain of *A. scabies* of tested pathogenicity was labelled "B" and used in all studies, except where otherwise stated.

EXPERIMENTAL RESULTS

Temperature Relations

The effect of temperature on spore germination has received attention by Shapovalov (15) and others, but a study of the influence of temperature on the pathogenicity of the strain of *A. scabies* used in these experiments seemed advisable.

Effect on growth and pathogenicity. Three strains of *A. scabies* (A, B and C) were used. Strains B and C were obtanied from S. A. Waksman, Rutgers College, N. J., and strain A was isolated by the writer and tested for pathogenicity. Duplicate cultures on nutrient agar slants were kept at the following temperatures: 7–8°, 10°, 13°, 16°, 19°, 22°, 24°, 26–27°, 29–30°, 32°, 35°, 37–38° C., and the growth recorded at 18-, 36-, 60-, 80- and 160-hour intervals. This experiment was repeated three times. All three strains reacted alike. The data for strain B are given in table 1.

TABLE 1.—*The effect of temperature on the growth of Actinomyces scabies, strain B*

Time in hours	Temperature in degrees Centigrade											
	7–8	10	13	16	19	22	24	26–27	29–30	32	35	37–38
	Rate of growth[a]											
18	0	0	0	0	0	1	2	4	5	4	2	0
36	0	0	0	0	1	3	4	4	6	5	3	0
60	0	0	0.5	1	4	5	6	7	8	6	3	1
80	0	trace	2	3	6	7	10[b]	12[c]	10	6	2	2
160	0	5	8	9	10	11	12[c]	12[c]	10	5	2	1

[a] Estimated value given to the relative progress of cultures after germination.
[b] Darkening.
[c] Dark.

[3] "Spores" is used in the widest sense because both conidia and mycelial segments function as reproductive structures.

It is evident from results that the range of temperature for growth increases with the time, and that germination and maturity take place sooner at the higher temperatures, with a corresponding decrease in vegetative growth. For instance, after 18 hours growth occurs between 22° and 35° C., while after 160 hours the range of temperature for growth extends from 10° to 38° C. Although good growth and maturity are attained at the lower temperatures in a longer time, the optimum point for greatest vegetative development and earliest maturity is 26–27° C. These results agree reasonably well with those of Lutman and Cunningham (9) who reported the optimum temperature to be 25° C.

Jones, McKinney and Fellows (6) stated that scab developed best at about 22° C., and that the amount decreased toward 11° and 30.5° C., where scab became negligible, as did also the yield of the tubers. They also suggested that the immediate effect of high temperature is more closely correlated with tuber development than with the growth of the pathogene. Scant growth of individual tubers, however, would not always explain the decrease of scab, because large scab pustules frequently form both on small and large tubers grown under field conditions. High temperatures may retard or prevent the growth of the scab pustule, subsequent to infection, but our knowledge of the process of tuber infection and scab development is insufficient to permit of conclusions.

The writer (14) obtained a maximum amount of scab in soils held at constant moisture content and temperatures of 14°, 17°, and 25° C.; and there was every indication that much scab would develop at at least two degrees lower or higher than 14° and 23° respectively. These results, with those of the present study, indicate that the growth of the pathogene in pure cultures, within the range of temperatures tested, is fairly well correlated with the amount of scab which develops. It might be concluded that temperatures lower than 12° C. will largely prevent scab development under field conditions and that periodic fluctuations between this point and 25° C. would not greatly affect the amount of scab. However, with various biologic and other factors, and a critical period for infection, temperature may prove to be more important in the field than it appeared to be under the controlled soil conditions tested.

Effect on viability. To determine the thermal death point of *A. scabies*, spores from a mature culture were soaked in water for thirty minutes and a very small portion of the spore suspension was put into 5 cc. of sterile water at the temperature under test. At ten- and twenty-minute intervals, portions were withdrawn and spread on cold agar plates to incubate. Repeated tests showed that spores germinated after having been exposed for twenty minutes at 83° but not after exposure at 85° C. They failed to germinate when exposed for ten minutes at temperatures higher than 90° C.

Since the spores of the scab organism resisted such high temperatures, it was decided to test the efficacy of the hot formaldehyde treatment. Experiment showed that scab spores on tubers were killed when immersed for two minutes in the hot formaldehyde solution (2 pints to 30 gallons of water) at 53° C. It also seemed desirable to test the efficacy of the acidulated mercuric chloride treatment for killing spores, because, if successful, its use would obviate the difficulty of the heating required with the formaldehyde treatment. The formula found effective by Cunningham (1) for killing sclerotia of *Rhizoctonia solani* was used. This method consists of soaking the tuber in a 1–1000 solution of mercuric chloride in water, to which has been added 5 cc. of commercial hydrochloric acid per liter. The tubers are kept moist for 24 hours after soaking. Experiments by the writer showed that this method was equally effective in killing spores of *A. scabies* and Rhizoctonia sclerotia.

Oxygen Requirements

Experiments were made to obtain more information on the relation of oxygen to the germination of the spores, the vegetative growth and fructification of *A. scabies*, because the pathogenicity of this soil fungus and the development of scab may be modified by the available oxygen in the soil.

Effect on germination and growth. A simple preliminary experiment suggested the strongly aerobic qualities of *A. scabies*. Six agar slants were inoculated with a spore suspension of the organism. Three tubes were sealed, and three were loosely plugged with cotton. The very faint growth which appeared after 30 hours in the sealed tubes was a striking contrast to the abundant development in those which were loosely plugged. No further increase occurred in the sealed tubes during 30 days. Apparently the available supply of oxygen had been exhausted during the first 30 hours. Another experiment, using the Büchner tube apparatus with alkalinated pyrogallic acid, demonstrated that the spores of *A. scabies* do not germinate in the absence of oxygen.

The relation of pure oxygen and nitrogen gas to the germination of the spores of the scab organism was also tested. These gases were allowed to displace the air in culture flasks, and then the flasks were sealed. No spores had germinated in 30 days in either gas, but, when exposed again to air, those which had been in pure nitrogen germinated and grew well. The spores which had been in oxygen were dead.

The effect of very limited and measurable amounts of oxygen on spore germination was also studied. Partial pressures, ranging from 0 to 29 inches, were obtained in test tubes containing agar slants which had been evenly inoculated with a spore suspension. Fresh inoculum was spread on the surface of the slants at the time the experiment was begun. The tubes

were fitted with rubber stoppers and glass tubes, and then sealed by heat after the air had been exhausted. By repeated experiment the results shown in table 2 were obtained. At zero partial pressure the spores required all the oxygen present in a 25 cc.-test tube to germinate and produce a very scant growth, while at a partial pressure of about 29 inches there was no visible sign of germination when examined microscopically. At a partial pressure of 28 inches short germ tubes appeared on some spores, but most of them did not germinate. The growth was very meager at a partial pressure of 27 inches. At partial pressures 25, 20, 15, 10, and 5 inches, a gradual increase in surface growth was evident over that obtained at a partial pressure of 27 inches, and increasingly higher percentages of spores also germinated at the lower partial pressures. The growth in the control tubes, which were loosely plugged with cotton, was in striking contrast to that at a partial pressure of 0 inches.

Effect on maturity. The relation of a limited amount of oxygen to vegetative growth, fructification, and especially to maturity (the darkening process of aerial hyphae) of *A. scabies* was investigated. Three inoculated petri dish cultures were placed in a large desiccator, a quantity of alkalinated pyrogallic acid calculated to absorb nearly all the oxygen was put at the base, and the cover sealed. White vegetative growth became evident in the control cultures in 24 hours and continued to increase, and on the fourth day the hyphae darkened rapidly. The test cultures grew as well as the controls up to the third day, but they failed to darken or to develop further during eleven days. When air was admitted, the retarded cultures grew quickly. In twenty hours they had darkened very noticeably, and by forty-eight hours were almost as dark as the controls. No detached spores and segments of mycelium were evident when the retarded cultures were examined on the eleventh day, but a later examination of the mature cultures revealed both the spores and segments common to ripe hyphae.

Relation of quantity of oxygen to partial pressure. While observing the germination and growth of *A. scabies* at the high partial pressures, it was suggested that the fungus might not be able to obtain the small quantity of oxygen left in the test tube; that is, the quantity of oxygen and not the partial pressure becomes the limiting factor to growth. No data on this point were found in the literature. Accordingly, a partial pressure of about 29 inches was obtained in two 25 cc. test tubes containing inoculated agar slants, and also in two 11,550 cc. bottles each containing one inoculated agar slant. The partial pressure was tested both in the large and small containers after ten days and found to have remained at about 29 inches. At this time, meager growth, similar to what had occurred in the test tubes at a partial pressure of 10–5 inches (see table 2) could be seen on the slant contained in each of the large bottles. The spores in the small test tubes at

the same partial pressure failed to germinate. The experiment was repeated three times with consistent results. These results suggest that this fungus is able to obtain practically all the oxygen left in a large volume, regardless of partial pressure. Therefore, it appears that quantity of oxygen, and not partial pressure, is the limiting factor to germination and subsequent development of *A. scabies*.

These studies show that *A. scabies* is very dependent on an abundant oxygen supply for quick germination and rapid vegetative development, and also for the ripening process, which means the darkening of the aerial

TABLE 2.—*The effect of various quantities*[a] *of oxygen on spore germination and growth of Actinomyces scabies*

Partial pressure in inches	Growth[b] and germination of spores after				Remarks
	24 hrs.	48 hrs.	72 hrs.	15 days	
29	0	0	0	0	No germination
28	0	0	0	0	Short germ tubes on a few spores
27	1	1	1	1	Extremely meager growth
25	2	2	2	2	More spores germinated; meager growth
20	2	2	2	2	*do*
15	2	2	2	2	Better germination
10	3	3	3	3	Faint growth
5	3	3.5	4	4	Faint growth
0	5	6	6	6	Better growth
Control[c]	25	75	95	100	Excellent ashen colored growth after 72 hours

[a] Obtained by partly exhausting the air from a 25 cc. space in test tubes; barometer reading 72.6 cm.

[b] Estimated relative value of growth at 26° C.

[c] Cultured in loosely plugged test tubes.

hyphae accompanied by easy disintegration of hyphal segments and spores for inoculum. No definite data have been secured relative to oxygen requirements in the soil, or the oxygen-supplying ability of soils of various types and moisture contents; but it seems quite probable that lack of sufficient oxygen is often an important factor in limiting sporulation, spore germination and vegetative development of the pathogene in the soil. This would then affect the ability of the parasite to cause infection and may help to explain why scab is not so prevalent in wet soils as in drier ones.

Moisture Relationships

From 1920 to 1923 the writer (13, 14) found that very little scab occurred when the soil was kept constantly very moist, but abundant scab

appeared in the same soil kept very dry. It was shown that the control obtained in the moist soil was not due to increased acidity, because the dry soil proved slightly more acid than when wet. It was thought that the pathogene might not sporulate as well in saturated soil air, or, possibly, the germination and growth might be delayed by the excess soil moisture around the spores.

Growth on solid media. Equal portions of spore suspension were spread on nutrient agar in petri dishes and cultured in various relative humidities, ranging from 1 to about 95 per cent. Saturated solutions of the following chemicals were used to obtain the relative humidities indicated: H_2SO_4, less than 1 per cent; LiCl, 7.16 per cent; $CaCl_2$, 25.94 per cent; NaOH,

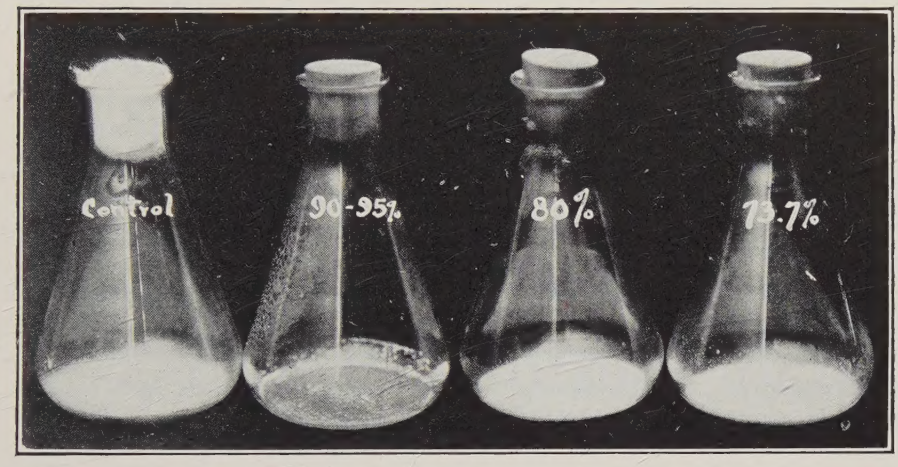

FIG. 1. Growth of *A. scabies* at various relative humidities. Note delayed germination and growth in second flask.

30.72 per cent; $AlCl_3$, 37.1 per cent; $Cu(NO_3)_2$, 45.7 per cent; NaCl, 73.7 per cent; $NaNO_3$, 80 per cent; K_2SO_4, 89.7 per cent; distilled water 90 to about 95 per cent. Owing to the surface condition of the agar when dried too quickly, no satisfactory data were obtained by this preliminary trial, although it was evident that excess water on the substrate delayed growth. In another experiment, saturated air was led successively through distilled water, and a saturated solution of sodium nitrate and sodium chloride. Between each of these solutions, and connected with them, was a 200 cc. flask containing 10 cc. of nutrient agar and 0.5 cc. of spore suspension over the surface of the medium. Within 15 hours vigorous growth appeared in the flask whose humidity was controlled by sodium nitrate, but not until after 60 hours in the flask whose humidity was determined by distilled water (figure 1). Final inspection showed that the cultures retarded by excess

moisture still lagged in development behind those getting an optimum start. This experiment was repeated a number of times. It is suspected that the film of water around the spores remained longer at the higher humidity than at the lower ones, possibly limiting the oxygen supply. It was repeatedly observed that dry spores put into water tend to remain on top, but, if the liquid is shaken frequently and the spores kept immersed, germination may be greatly delayed. If left, however, the spores on top germinate, while those immersed usually remain dormant. It was found that optimum conditions, good vegetative growth can be secured in 24 hours, spore suspension evenly over the surface of agar slants in loosely plugged test tubes, and incubating them in an upright position at 27° C. Under optimum conditions, good vegteative growth can be secured in 24 hours, and a fair degree of maturity (as indicated by the dark color of the aerial growth) in 48 to 60 hours, but this may be greatly postponed by less favorable conditions, even at optimum temperature.

Viability of spores. Studies were made to determine the effect of relative humidities at various temperatures on the viability of the spores of *A. scabies.* A small amount of the aerial hyphae from mature cultures was placed in small sterile phials. These were lightly plugged and placed in sealed jars. Relative humidities of approximately 95, 80, 46, 37, 7 and 1 per cent were obtained by using distilled water, saturated solutions of sodium nitrate, copper nitrate, aluminium chloride, lithium chloride, and concentrated sulphuric acid, respectively. A series of each was incubated at 30°, 26° and 12° C., respectively. Viability tests were made each month. After six months, the spores germinated well, regardless of temperature or humidity.

Soil moisture and increase of inoculum. The amount of inoculum in the soil during the susceptible period of the tuber is an important factor influencing the amount of infection. The soil dilution and plate method was used to determine the relative effect of moist and dry soils on the increase of inoculum. Eight grams of finely-sifted, oven-dry soil was put into a 200 cc. flask and sterilized with dry heat. Five cc. of spore suspension was put into each flask, and after the moisture in the inoculum had diffused, the soil was thoroughly mixed, then various additional amounts of sterile water were pipetted into the flasks and distributed as evenly as possible. The culture flasks were in triplicate in each series. Provision was made for aeration, and the soil cultures set to incubate in a high humidity at 26° C. for 30 days. Dilutions were made by adding enough sterile water to the flasks so that each would finally contain 100 cc. The contents of each flask were then emptied into 900 cc. of sterile water. After shaking and allowing the contents to settle for 30 seconds, a 100 cc. portion was withdrawn from a uniform depth and introduced into another 900 cc. of water. Further

dilutions of 10–90 cc. were made, and a 1 cc. portion of the final dilution was pipetted into each of four culture plates. This method of dilution was used to reduce the error arising from the possible unequal distribution of the inoculum added to the culture flasks, and the very high probable error that was found by repeated trials to exist when only 1 cc. portions were withdrawn from the first dilution. Even this method leaves much to be desired, as the probable error is still high.

The results shown in table 3 indicate that the inoculum failed to increase in the driest soil (6.25 per cent), but at 10, 15, 21.2 and 27 per cent soil moistures it continued to increase until it reached about five-fold its original amount. A sudden decrease is observed at 33.7 per cent soil moisture. Possibly this is on account of the lack of fructification, caused by the hyphae in the very moist soil retaining their vegetative state and failing to break up into segments. The increase at 37 per cent soil moisture is abnormal since in this excessively wet soil the spores were carried by free water to the surface, where they germinated, and where sporulation later occurred.

TABLE 3.—*The effect of soil moisture on increasing the inoculum of Actinomyces scabies cultured in a sandy loam*

Soil moisture in per cent	6.25	10	15	21.2	27	33.7	37
Average no. colonies obtained from diluting 80 grams soil	7.1	20.2	31	38	34	17	45[a]
Per cent increase over control (7.1)		184.5	336.6	435.2	378.8	139.4	533.8

[a] This abnormal increase is the result of surface growth on the excessively wet soil.

Soil moisture and scab development. According to the results of the previous experiment and the behavior of the pathogene on nutrient agar, favorable conditions for production of abundant inoculum, and for subsequent growth, would not be found either in excessively dry or in very moist soil but in one of intermediate moisture content. However, the writer (13, 14) found that a maximum amount of scab may develop in a very dry soil.

In one instance tubers developed to maturity in soils having about 4 per cent of moisture by weight (oven-dry basis). The water was led by a tube below the surface soil, in which the tubers formed, to the roots of the plant. It was thought sufficient moisture transpired from the tuber in so dry a soil to enable the spores adhering closely to the surface, or about the lenticels, to germinate, and that the germ tubes grew toward the source of

moisture. Perhaps increased infection of the tuber in dry soils may be due to a hydrotropic response, or the lenticel structure and hydrotropism both may affect it. When once established on the host, the pathogene would develop regardless of the lack of soil moisture, and a dry soil would allow good aeration. Lutman (8) states that scab may originate at any place on the surface of the tuber, and that it most frequently occurs at the lenticels. From extensive observation, the writer believes that the lenticels are the principal infection courts, and are the centers from which the typical scab pustules develop. It is common knowledge that the proliferation of the lenticels of potatoes is increased in wet soils, and it is quite possible that there is a close correlation between early lenticel growth in dry soils and susceptibility to the scab pathogene.

The possibility of the growing tuber exerting a chemical stimulus on the spores of the scab organism, or on the germ tubes, was not overlooked. From repeated experiment there was no indication that the tissue of young or old tubers, or of potato leaves, stimulated the germination of the spores of *A. scabies.*

The writer (13,14) twice reported a critical period of susceptibility to scab infection. This occurred during the first ten days of tuber development. With a critical period for infection, the relation of soil moisture to scab development becomes very important. Young tubers may escape infection during this period if excessive soil moisture or other unfavorable factors inhibit the growth of the pathogene, and, likewise, an effective means of control must exert its greatest influence at this time.

Relation of Soil Reaction to Germination and Growth

Gillespie (4) concluded from a study of several cultures of *Actinomyces scabies,* that a pH of 5.2 is about the lower limit for growth. Slight development occurred in few instances at pH 4.8.

The effect of hydrogen-ion concentration on the growth and spore germination of the strain used in these experiments was determined. A wide range of pH was obtained by adjusting Czapek's synthetic dextrose-nitrate solution, as recommended by Karrer and Webb (7). Twenty cc. of sterile solution thus made up were measured into 200 cc. pyrex flasks, loosely plugged, then 1 cc. of spore suspension was added, and the flasks set to incubate at 26° C. Electrometric determinations were made of the initial reaction of each series, with and without inoculum, and this was repeated in ten days in the acid series, and in 48 hours in the alkaline series, in order to note any change in reaction. The solutions containing inoculum soon became alkaline, depending on the rate of growth. Notes were taken on the time required for growth to appear, its relative progress and final develop-

ment. Each series was in triplicate, and the whole experiment was repeated three times. The results are summarized in table 4.

Evidently pH 5.36 is about the limiting acidity for germination of the strain of *A. scabies* used in the test, since microscopic examination at the end of 20 days showed no evidence of spore germination at this reaction. Possibly other pathogenic strains of *A. scabies* have a different limiting pH value for germination. A few slow growing colonies appeared on the seventh day at pH 5.56. At pH 6.95 to pH 9.82[4]–9.05, visible growth occurred in 48 hours. For the first three days there was a constant tendency for earlier and better growth above than below pH 8.30, but after this period the cultures growing in the media with initial pH ranging from 6.48 to 8.68

TABLE 4.—*The estimated growth progress of Actinomyces scabies, at 26° C, on Czapek's solution at different hydrogen-ion concentrations.*

Culture	pH value of medium		Growth progress at various day intervals					
	Initial	After 10 days	2	3	4	5	7	20
1	4.52	4.50	0	0	0	0	0	0
2	4.70	4.70	0	0	0	0	0	0
3	4.87	4.86	0	0	0	0	0	0
4	5.21	5.20	0	0	0	0	0	0
5	5.36	5.37	0	0	0	0	0	0
6	5.56	5.83[b]	0	0	0	0	tr.	4[c]
7	5.69		0	0	tr.	1	2	6
8	6.08		0	1	3	7	8	8
9	6.48		0	1	4	8	10	10
10	6.85		0	2	6	9	10	10
11	6.95		tr.	2	6	9	10	10
		After 48 hours						
12	7.16		tr.	2	7	9	10	10
13	7.56	7.51	tr.	3	7	9	10	10
14	7.76	7.66	tr.	3	7	9	10	10
15	8.38	8.30	1	4	7	9	10	10
16	8.68	8.51	2	6	8	9	10	10
17	9.31	8.80	2	6	8	9	9	9
18	9.43	8.88	2	6	8	8	8	9
19	9.82	9.05	2	6	8	8	8	8
20	10.21	9.34	0	0	2	4	5	7

[a] 0 = no growth; tr. = trace; 1 = faint; 2 = plainly visible; 4 = fair; 6 = good; 10 = excellent.

[b] Determination made from growing culture.

[c] Few well developed colonies.

[4] The first figure is the initial reaction, the second that obtained after 48 hours.

seemed to have made more vegetative growth and to have fructified more abundantly than those above pH 8.68. Germination was slow and final growth poor above pH 10.2–9.34. No attempt was made to determine the maximum pH for germination because of the difficulty of holding the high alkaline solutions constant and the probable effect on growth of the large amount of potassium hydroxide. It would appear, however, that this point is not far from pH 10.

Available food, soil, aeration, moisture, and other biological factors, as well as soil reaction, must influence the amount of inoculum in the soil, and the amount of scab which develops. However, it does not appear that a soil reaction which is the limiting point for germination of the inoculum will completely inhibit development of scab. This might be because the growing tuber has a reaction favorable to germination and growth of *A. scabies*. Weiss and Harvey (19) found the reaction of the potato tissue to vary from pH 6.5 to 6.61. Consequently, the acidity about the closely adhering inoculum might be decreased enough to allow germination of the spores and infection of the host. If so, subsequent development would be largely determined by the reaction of the host tissue. Possibly this suggests why Martin (10) obtained scab on 20.6 and 89.6 per cent of the tubers in a soil of pH 4.2 and 4.8, respectively.

Weiss and Harvey found that tissue affected with potato wart was more acid (pH 6.0) than the adjacent sound tissue (pH 6.5). The writer determined electrometrically the reaction of scab pustules. Very scabby young potatoes grown in a soil having a reaction of about pH 5.4, were washed in distilled water, the scab pustules removed, finely pulverized, and the material diluted with distilled water. Sound tissue was treated likewise, and determinations made as soon as possible. The pH of the sound tissue was about 6.5, while that of the scab pustules was about 7.2. This increase in alkalinity agrees with what happens in liquid cultures and supports the previous suggestion that the development of *A. scabies* on infected tubers is largely independent of soil reaction.

The Relation of Green Crops to the Control of Potato Scab

The popular practice of plowing down green crops, particularly rye, to reduce the severity of scab is based largely on the assumption that decomposition of the green material increases soil acidity. Since consistent evidence for such belief seemed to be lacking, the writer tested the efficacy of the method in a well checked field experiment.

The effect of green rye plant material on soil reaction. A small, nearly level, thoroughly infested plot of fertile silt loam, with an initial pH ranging from 4.91 to 5.4, was chosen for the test. Twelve rows, of 12 hills each, were planted, test rows and controls alternating. Two and one half pounds

of succulent green material was mixed with the soil, and this put back into the excavations, which were 14 inches in diameter and 7 inches deep. This proportion of green material amounts to about 50 tons per acre. The soil in the control hills was forked to make it as loose as that under test. A disinfected set of the cobbler variety was planted 5 inches deep in each hill. The soil about each plant was not disturbed during the growth period. The rye was almost completely decomposed by harvest, 80 days after date of planting (May 24). The reaction of the soil, taken from representative hills at depths of 2, 4 and 6 inches, was determined electrometrically on June 19, July 2, and July 22. Samples were taken from the same hills each time, so that any change in soil reaction resulting from the decomposition of the green material might not be confused with soil heterogeneity. One hundred and thirty-two samples, of which 24 were checks, were tested, and three determinations made from each sample.

The object of this experiment was to note any change in soil reaction caused by the green rye during the early growth period of the potato crop, and particularly during the critical stage for scab infection. Therefore the reaction of the soil was not determined after 58 days. The data indicated

TABLE 5.—*The effect of varying amounts of green rye on the soil reactions and on the relative number of bacteria and fungi*

Parts of rye to soil	Interval after which pH was taken					Initial pH	Maximum pH increase	No.[a] colonies	
								Bacteria[b]	Fungi[c]
	Series 1								
	10 days	20 days	28 days	36 days	78 days				
	pH	pH	pH	pH	pH				
1–5	8.99	7.56	7.25	7.10	7.04		1.82		
check	7.22	7.20	7.13	7.14	7.14	7.17			
	Series 2								
	7 days	15 days	33 days	75 days					
	pH	pH	pH	pH	pH				
1–3	8.82	8.22	5.82	5.07			3.60	200	70
1–7.5	7.70	7.49	5.24	4.98			2.48		
1–10	7.37	5.76	5.37	4.97			2.15	134	33
1–15	6.89	5.73	5.24	5.24			1.67		
1–20	6.67	5.51	5.27	5.22			1.45	16	26
check	5.31	5.25	5.22	5.19		5.22			

[a] In millions per gram of rye soil mixture, oven dry.

[b] Dilutions made after 20 days.

[c] Dilutions made after 10 days.

a slight decrease in soil acidity. This conclusion agrees closely with that of Stephenson (16) who found that in nearly every case the acidity was decreased where soy beans, oat straw, green rape, and blood were used alone and in combination in the soil. Oat straw had the least effect in decreasing the acidity, while the highly nitrogenous material had the greatest.

Another study was made in the laboratory to determine the effect of the decomposition of green rye plants on soil reaction. A quantity of leaves from green rye plants was finely ground. This was mixed in varying amounts with finely sifted, unsterilized garden soil, then moistened with distilled water and put into loosely plugged liter flasks. All samples were approximately at optimum moisture content when completed, and the soil was loose in the flasks. The initial reaction of the soil, and also of the green material used, was determined electrometrically. The flasks were kept at room temperature and the soil reaction determined at intervals during 80 days. The number of fungi and bacteria developing in the soil of the various flasks was obtained by the usual dilution-plate method, and a comparison made on an oven-dry basis per gram of soil.

An analysis of the results in table 5 shows that the pH is decidedly increased in all the soils mixed with green rye-plant material, especially during the first ten days, and that this sudden rise is succeeded by a slow but steady decline during the second period. After about 30 days the pH values approach a level approximating the initial reaction. This is especially noticeable in the soils containing the smaller proportion of plant material. The increase in pH is, in general, proportional to the amount of plant material added to the soil, and varies from 1.45 to 3.60. In most cases, even after 30 days, this advantage is maintained. After 30 days, and continuing to 75 days, all the samples return to the initial reaction and, when the last determinations were made, only those samples receiving the larger proportions (1–3, 1–7.5, 1–10) of rye were slightly more acid than the untreated soils.

Since the determinations were made on the rye-soil samples after fermentation began, the increase due to the reaction of the rye itself was unknown; therefore a separate experiment was necessary to obtain this information. The initial pH of the green rye was 6.82, and that of the soil 5.12. The data show that the pH of the rye-soil mixture was increased very slightly by adding the green rye. The largest proportion of rye to soil (1–5) increased the pH 0.36, while the 1–10, 1–15, 1–20 proportions gave increases of 0.20, 0.18 and 0.17, respectively. After an 8-day incubation period the increases in pH of these same mixtures were: 1–5, 1.71; 1–10, 0.94; 1–15, 0.80; 1–20, 0.71. Apparently, then, nearly all the increase in pH of this and the previous test was the result of fermentation.

Two suggestions are given for the consistent increase in pH of the rye-treated soils; either the rye may favor certain microorganisms which decompose it and liberate ammonia for a time, or certain alkaline by-products of metabolism may be secreted by the fungi and bacteria themselves. Doryland (2) found that nitrification begins when the more easily available carbohydrate energy is exhausted, and states that the ammonia and nitrates which have been accumulated by the decay-producing organisms during this period are later available for nitrification. Waksman (18) states that the molds are not nitrifiers but they actively decompose organic matter, taking energy first from the available carbohydrates and then from protein molecules, in which case ammonia is liberated. He also points out how ammonia may be liberated upon autolysis of the mycelium of these molds. Neller (12) found that certain pure cultures of bacteria caused the accumulation of much more ammonia than some of the more active soil fungi (*Aspergillus niger*, *Trichoderma* sp., and *Zygorhynchus* sp.) and that there was an indication of symbiosis in mixed cultures of certain soil bacteria in increasing the accumulation of ammonia.

It is possible that the large amount of carbohydrate energy in the rye permitted abundant and quick growth of microorganisms, with a corresponding nitrate depletion of the soil, and that, upon the sudden exhaustion of the carbohydrate energy supply and the consequent setting in of the nitrification process, certain more favored bacteria and fungi actually cause the liberation of ammonia.

It was suggested that the increase in pH might be due to the alkaline by-products secreted by one or more predominating species of microorganisms. It has been shown that *A. scabies* greatly increases the pH of the culture media tested. It also is well known that other microorganisms produce acids and alkalies, depending on the chemical nature of the substrate.

A plate count of the microorganisms was made from dilutions of the soil of Series 2 (see table 5). In the dilutions made 10 days after mixing the rye and soil, the number of fungi on the plate made the count of the bacteria too uncertain to record, but, when made after 20 days, the plates were fairly free from fungi, and bacteria predominated. A heavy growth of molds (*Mucor racemosus* appeared to predominate) occurred in all flasks containing the rye-soil mixture. This took place two days after mixing; and in ten days it had declined, the soil taking on a moist, slimy appearance, which indicated bacterial activity. No further change was noted and no more soil dilutions were made. It is suggested that the fungal-bacterial succession referred to here may be associated with the rise and decline of pH noticed in these rye-treated soils.

The effect of green rye plant material on scab development. Figure 2 shows the amount of scab developing on the tubers in rye-treated and untreated soils of the field. Each pile represents the complete yield from a row, and these are arranged as they grew—treated and untreated rows alternating. No difference between the test lots and the controls can be noticed: all are badly scabbed. These results differ from those of Millard (11), who obtained good control by a liberal application of green rye; and, since the data on soil reaction could not explain the results, a preferential food theory was offered. This theory assumes that the scab pathogene, being normally a soil saprophyte, prefers green rye to the living potato tissue. The hypothesis may be tenable. The writer advances another possible explanation, and this will be referred to in connection with the antibiosis of certain bacteria and fungi to *A. scabies.*

FIG. 2. The piles of tubers marked X are from soil (pH 5 to 5.4) treated with 50 tons per acre of green rye; remainder from untreated soil. Note uniformly large amount of scab in all piles.

The data from the field experiment showed that the decomposition of 50 tons of green rye per acre caused no noticeable increase in soil acidity during the 58-day period and failed to prevent the development of scab. The laboratory experiment showed that it required a much larger quantity than this to increase the acidity of the same soil, even slightly, at the end of 75 days. It is interesting to recall that the initial reaction of this soil varied from pH 5.0 to 5.4, which is very near the limiting reaction for growth of *A. scabies* in pure culture. The acid-producing effects of green rye on other soil types was not tested. However, these results strongly suggest that an abnormally large amount of rye would be required to increase the acidity of less acid soils to an effective point for scab control. Therefore it is the

opinion of the writer that the use of green rye as a practical means of increasing the soil acidity for the control of potato scab is not feasible. If control can be obtained by its use in some soils, it may be that certain microorganisms of the greatly increased soil flora are antibiotic to *A. scabies.*

Cultural Relations of Certain Bacteria and Actinomyces scabies

While testing the stimulatory effects of potato leaf- and tuber-tissue on the germination of the spores of *A. scabies,* it was frequently observed that the spores failed to germinate on certain slides overrun with bacteria. Further examination showed that the lack of germination was often associated with the kind of bacteria present, therefore possibly a result of toxicity or a too highly acid condition.

Effect of mixed cultures on the pH of the substrate. To study the effects of association of bacteria and *A. scabies* in culture, a number of pure cultures of bacteria were secured from various sources, chiefly from the soil. These were numbered as represented in table 6. Culture 21 is *Bacterium fluorescens;* 28, *B. mycoides;* 24, *B. cereus.* These were obtained from H. J. Conn, New York Agricultural Experiment Station. Culture 22 is *B. vulgatus;* 23, *B. megatherium;* 25, *B. mycoides;* 27, *B. mesentericus,* all of which were furnished by O. B. Williams, University of Texas. Czapek's dextrose nitrate solution was used for a medium. This was sterilized and adjusted to the required pH values by the addition of one-tenth normal potassium hydroxide. Pyrex flasks were used, and to each was added 20 cc. of the medium, and inoculum consisting of 0.1 cc. of a spore suspension of *A. scabies,* and the amount of bacteria adhering to the small loop of a transfer needle. Each culture was made in triplicate and incubated at 26–27° C.

The results given in columns 2 and 7, table 6, indicate that nearly all the strains of bacteria are strong acid producers. The reactions listed in column 2 give an idea of the acid-producing ability of each pure culture of bacteria when grown alone. Cultures 21 and 29 are fairly strong acid producers, although in Series 1 the time during which they were incubated is too short to ascertain the final effect in this respect. The reactions in column 6 indicate the strong alkali-producing ability of *A. scabies* during six days, when the initial pH of the medium is 7.3. This quality is also shown, in columns 9 and 11, in those cultures where the Actinomycetes are growing vigorously, apparently having secured control by neutralizing the acid from the bacteria. Evidently *A. scabies,* when grown alone or with certain bacteria, grows and fructifies exceptionally well at pH 8.0 to 8.7. Most of the cultures of bacteria allow a good final growth of *A. scabies* if the initial pH of the medium is sufficiently high. Bacterial cultures 11, 15, 21 and 29, however, always prevent the growth of the Actinomycetes. In the case of

cultures 11, 21 and 29, the growth is prevented by a too acid condition of the medium, *i.e.*, pH 4.23, 4.43 and 5.29 respectively. These cultures have reduced the pH from 7.3 to these low values in six days, while most of the other strains allowed the Actinomycetes to increase it to about 8.7. Culture 15 is outstanding, because it always prevents the growth of *A. scabies*,

TABLE 6.—*The effect of pure cultures of bacteria growing alone, and also with A. scabies, on the pH value of Czapek's solution*

Culture no.	Series 1					Series 2				
	Bacteria initial pH 7.3	Bacteria and *A. scabies* initial pH 7.3				Bacteria initial pH 6.87	Bacteria and *A. scabies* initial pH 5.7			
		Growth: after 3 days	pH	Growth: after 6 days	pH		Growth: after 6 days	pH	Growth: after 11 days	pH
1	6.53	Good	7.41	Ex.[a]	8.67	.....	.....	.....	.....	.....
2	6.44	do	7.68	do	8.72	.....	None	5.02	None	5.41
4	6.12	do	6.48	do	8.75	.....	.....	.....	.....	.....
5	6.13	do	7.12	do	8.73	.....	None	4.91	None	5.59
8	6.61	do	7.33	do	8.72	.....	.....	.....	.....	.....
10	6.99	do	7.67	do	8.69	.....	.....	.....	.....	.....
11	6.05	Trace	4.34	None	4.23	.....	None	4.77	None	4.57
12	6.86	Good	7.39	Ex.	8.73	.....	do	5.40	Ex.	8.04
13	5.67	do	7.80	Good	8.72	.....	.....	.....	.....	.....
15	6.78	None	6.63	None	7.28	.....	None	7.35	None	7.29
16	6.44	Trace	6.44	Ex.	8.72	.....	Trace	4.89	do	4.22
17	6.61	do	6.95	do	8.59	.....	None	4.57	do	4.60
19	6.30	Good	7.14	do	8.57	5.51	do	5.32	Good	8.14
20	6.89	do	7.17	do	8.57	.....	.....	.....	.....	.....
21	6.71	None	5.11	None	4.43	4.30	None	4.73	None	4.80
22	6.61	Good	7.49	Ex.	8.72	.....	.....	.....	.....	.....
23	6.81	Trace	5.43	Faint	7.36	.....	None	4.92	Edge	5.41
24	6.53	Good	7.61	Ex.	8.72	6.16	do	5.29	do	6.41
25	6.71	do	7.21	do	8.72	6.14	do	4.81	None	4.73
26	6.64	do	6.97	do	8.70	.....	.....	.....	.....	.....
27	6.69	Trace	7.39	do	8.79	5.66	None	4.89	None	5.03
28	6.53	Good	7.26	do	8.89	5.92	do	4.72	do	4.72
29	6.66	None	5.26	None	5.29	5.34	do	4.83	do	4.64
30	6.99	Edge	7.56	Pel.[b]	7.80	.....	Pel.	7.35	Edge	7.63
31	6.71	Fair	7.22	Fair	7.46	.....	None	5.27	Ex.	8.04
32	7.05	Good	8.52	Ex.	8.72	.....	.....	.....	Pel.	6.07
33	6.66	do	8.01	do	8.72	.....	Pel.	7.06	Edge	7.70
Check	7.70	do	8.51	do	8.72	.....	Fair	7.06	Ex.	8.40

[a] Excellent.

[b] Pellicle.

although it does not reduce the pH below a point favorable for growth of the fungus. When the initial pH of the medium is low (5.7), as in Series 2, many of the cultures of bacteria retard or prevent the growth of *A. scabies* by quickly increasing the acidity of the substrate, but some do not produce acid fast enough to accomplish this. This competition between the bacteria and the Actinomycetes makes the final outcome uncertain, as is shown by cultures 2, 5, and 23, in columns 9 and 11.

Further examination of table 6, Series 1, shows that some strains of bacteria increase the acidity of the medium much more when cultured with *A. scabies* than when alone. This action is well illustrated by cultures 11, 15, 21 and 29, in figure 3 and particularly by cultures 11, 21 and 29. The decrease in pH, as a result of association, ranges from 0.95 to 1.71. A parallel case is reported by Neller (12), who found that *Bacillus mycoides* and *B. vulgatus* produced more ammonia in mixed cultures than when alone, and that the same thing was true of the production of carbon dioxide by *B. subtilis* and *B. vulgatus*. Other instances of increased activity arising from the association of certain microorganisms are known.

Toxicity and compatibility in mixed cultures. Grieg-Smith (5) states that certain white-growing colonies of Actinomycetes secreted some product very toxic to *Bacillus vulgatus, B. mycoides* and also to *Penicillium* spp. and *Aspergillus* spp., and that the dark-growing[5] Actinomycetes were only feebly toxic to *B. vulgatus*. The writer has observed that *A. scabies* is, in general, very sensitive to the secreted products of many molds and bacteria, some of which prevent its growth. *Rhizopus* spp. have been consistently very toxic. Species of *Penicillium* and *Aspergillus* usually appear to be toxic to *A. scabies,* but this condition often develops later into one of compatibility, especially on solid media. Some of these cases of toxicity are probably associated with competition for food and with an unfavorable substrate reaction. The competition for favorable substrate reaction at the minimum point for growth is well illustrated by *A. scabies* and bacterial cultures 19 and 24, Series 2, table 6, and in figure 3, part 2. Bacterial culture 15 evidently secretes another kind of toxic substance, which, while not too acid, prevents the growth of *A. scabies.*

Further investigation of the association of *A. scabies* and the various strains of bacteria cultured in solution (table 6) was made on solid media. Potato dextrose agar slants were evenly inoculated with 0.2 cc. of spore suspension of *A. scabies* and then inoculated for 24 hours. By that time growth was quite evident. Then 0.2 cc. of sterile distilled water was added to the very bottom of each tube, and this was inoculated with a small amount of bacteria adhering to the loop of a transfer needle. Care was taken to add

[5] *Actinomyces scabies* belongs to this group.

nearly equal quantities of the bacterial inoculum to the water at the bottom of the tube only, and to keep the tubes upright. Striking differences in

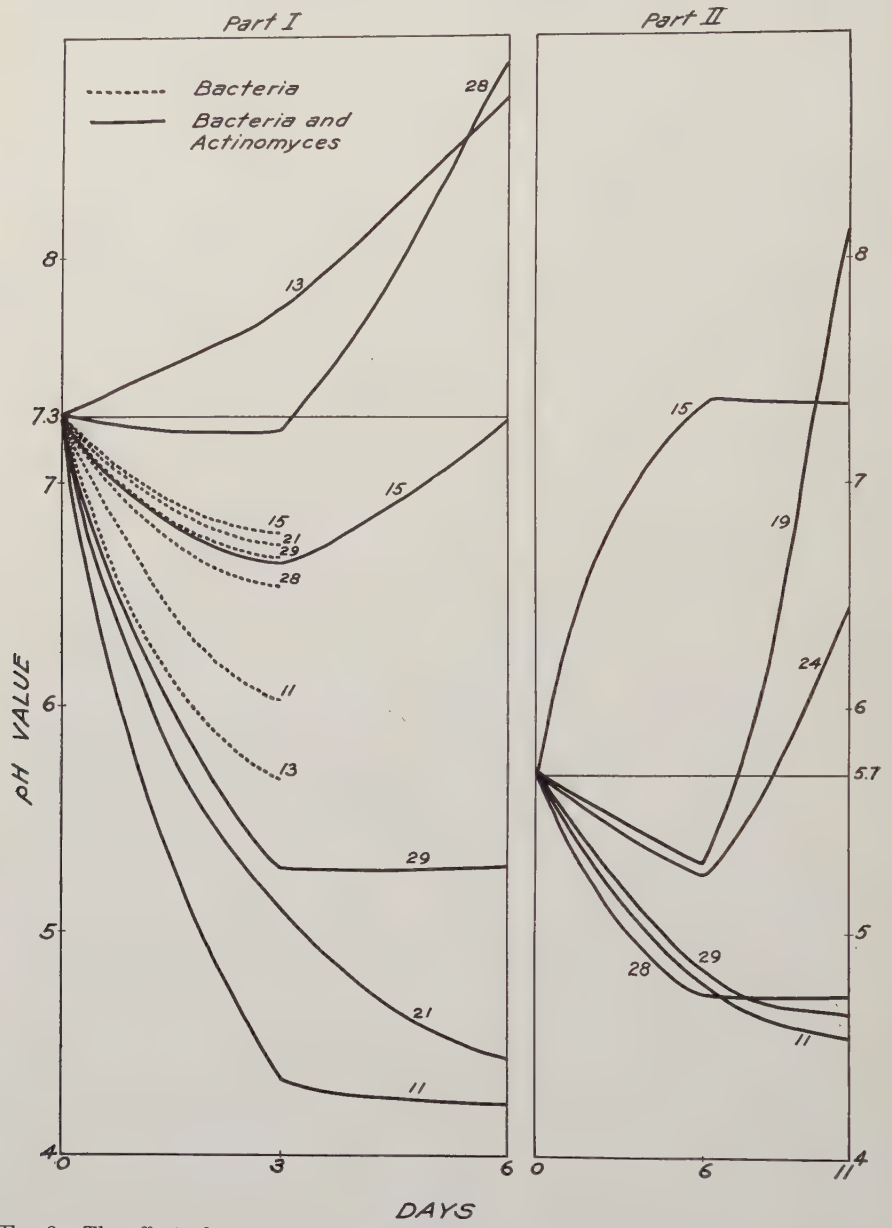

FIG. 3. The effect of pure cultures of bacteria growing alone and also with *A. scabies*, on the pH value of Czapek's solution. (Each culture is designated by the number just above the pH curve; the numbers correspond with those in table 6.)

growth were evident in a few days. A number of the strains seemed more or less compatible. As happened in the liquid media, bacterial strains 11, 15, 21 and 29 were sufficiently toxic to *A. scabies* to destroy the growth after it was visible. Cultures 2, 5, 12, 28 and 30 also were very toxic, although they had not shown this in the liquid media. The phenomenon is not unlike the activity of a lytic principle.

It is quite possible that the same degrees of compatibility between organisms and the same toxicity demonstrated on artificial media may also occur naturally in the soil. This is a problem of the biological balance in the soil and therefore may be of particular importance in determining the pathogenicity of *A. scabies.*

SUMMARY AND CONCLUSIONS

1. A study has been made of the effect of temperature, moisture, aeration, and substrate reaction on the germination, growth and fructification of *Actinomyces scabies,* with particular reference to its pathogenicity. The relation of green rye to the control of potato scab has been studied, and its effect on soil reaction and on the increase of soil flora has been observed. A brief study has been made of some cultural relations between *A. scabies* and some common soil bacteria.

2. *A. scabies* grows within a wide range of temperature (8 to 38° C). Good growth and maturity occur between 13° and 32° C., and the optimum temperature is about 27° C. Therefore, under average field conditions in most potato growing areas, it appears that temperature, as it affects host and pathogene only, cannot be a very important factor in the scab problem. The spores survive temperatures up to 90° C. (moist heat) for ten minutes.

3. *A. scabies* is a strong aerobe. The spores will germinate with an extremely small supply of oxygen, but a large amount is required for subsequent development. Maturity, as indicated by dark aerial hyphae, will not take place in the absence of oxygen. Amount of oxygen, not partial pressure, is the limiting factor for germination and growth.

4. It was found that the germination of spores of *A. scabies* on nutrient agar was greatly retarded by a lagging film of excess water. The inoculum of *A. scabies* appeared to increase best at a soil moisture content about optimum for plant growth.

5. The limiting acid reaction for germination of the spores of the strain of *A. scabies* used was found to be about pH 5.3. Germination occurred most quickly at about pH 8.5, and an optimum development took place at this point. Severe scab may be expected in soils ranging from a strongly alkaline reaction to at least pH 5.4, owing to the higher pH of the tuber and a strong tendency of the pathogene to make its habitat (scab pustule) alkaline.

6. Green rye plants, applied in the field at the rate of 50 tons per acre, showed no tendency to reduce scab in a thoroughly infested soil of pH 5 to 5.4; and there was no noticeable increase in the soil acidity during a 58-day period.

7. In the laboratory there was a very slight increase in acidity of the rye-treated soils after 75 days, but only in those receiving large proportions (1–3, 1–7.5, 1–10) of green rye. These results, and those obtained in the field, indicate that the use of green rye does not control potato scab by increasing the soil acidity.

8. When pure strains of bacteria and *A. scabies* were cultured in the same solution, certain strains of the former made the solution too acid for the germination of the latter. Certain cultures of bacteria produced more acid when grown with *A. scabies* than when grown alone.

9. A strain of bacteria isolated from the soil, and cultured in Czapek's solution, inhibited the development of *A. scabies* by some means other than excessive acidity.

10. Nearly all the bacterial strains used in this study made conditions more or less unfavorable for the growth of *A. scabies* on a potato-dextrose agar.

11. When scab is controlled by green rye crops on some soils, it is suggested that the antibiotic qualities of certain predominant soil microorganisms influence the development of *A. scabies*.

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